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(54) **Nematocides**

Nematozide

Nematocides

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## Description

**[0001]** The present invention relates to the use of loquat kernel to kill, inactivate or repel nematodes, and in particular as a nematicide, i.e. a composition that kills nematodes, and more particularly as a nematicide that selectively kills plant parasitic nematodes. The invention also relates to methods of killing nematodes and to nematicide compositions and their methods of manufacture.

**[0002]** Nematodes are a type of worm like organism. There are different trophic groups of nematodes: fungus feeding, bacteria feeding, predatory and plant parasitic. Plant parasitic nematodes, such as root knot nematodes, are pests because they cause plants to stunt, wilt and/or yellow, often with galls developing over the roots of the plant, due to the feeding of the nematodes on the roots of the plants. Plants that are particularly vulnerable to plant parasitic nematodes include banana, capsicum, carrot, celery, cucurbits, beans, egg fruit, ginger, grape, kiwi fruit, strawberry, lettuce, carnation, chrysanthemum, rose, papaw, passion fruit, pineapple, peach, pumpkin, nectarine and tomato.

**[0003]** There is therefore a need for nematicides to control plant parasitic nematodes; in particular for nematicides that selectively destroy plant parasitic nematodes over other nematodes.

**[0004]** The loquat (*Eriobotrya japonica*) is a fruit of the family Rosaceae. The family Rosaceae also includes the fruits of the genus Prunus, which include almonds, apricots, peaches, plums, nectarines and cherries. The loquat is also known as the nispero and references hereinafter to the loquat should therefore be read accordingly.

**[0005]** The loquat is classified as being a fruit of the type "pome"; pomes are fleshy fruits having a thin skin, which consist of enlarged receptacle tissue enclosing the ovary and seeds. Other pomes include apple, pear and quince, although the seeds of the loquat are very large (ca 1-3cm diameter) compared to apple or pear seeds.

**[0006]** The leaves, kernel and fruit of loquat are known for their medicinal uses; in particular the fruit is said to act as a sedative and to relieve coughing and vomiting whilst its leaves and kernel contain laetrile, which is believed by some to have anti-cancer properties. There has not, until now, however, been any teaching regarding the use of the loquat in pesticidal applications.

**[0007]** The present invention provides the use of loquat kernel to kill, inactivate or repel nematodes. In a particularly preferred embodiment, the use of loquat kernel as a nematicide is provided.

**[0008]** It has been identified by the present inventors that the loquat kernel can effectively be used in the killing, inactivation, and/or repulsion of nematodes.

**[0009]** In particular, the invention provides the use of loquat kernel as a nematicide for plant parasitic nematodes, preferably as a selective nematicide for plant parasitic nematodes.

**[0010]** As can be seen from the Examples, selective killing of plant parasitic nematodes can surprisingly be achieved by the application of loquat kernel.

**[0011]** In particular, the use of loquat kernel as a selective treatment for nematodes over fungi is provided. As can be seen from the Examples, loquat kernel selectively acts as a nematicide; it does not act as a fungicide.

**[0012]** The kernel may be used in any suitable form; in particular it may be provided in ground, chopped, freeze dried or spray dried form or in the form of an extract. When in the form of an extract any suitable solvent may be used, for example an aqueous solvent, such as water, or an organic solvent, such as ethanol, acetone or dichloromethane. In one embodiment, the kernel is used in the form of an extract in water. In an alternative embodiment, the kernel is used in the form of an extract in ethanol.

**[0013]** In one embodiment, all soluble components from the loquat kernel are used; in particular all water-soluble components and/or all ethanol-soluble components from the loquat kernel may suitably be used.

**[0014]** The loquat kernel may be used in the form of an extract, preferably an extract in water, that has a zero or substantially zero concentration of volatiles; in particular the extract may suitably have had all or substantially all volatiles removed.

**[0015]** In an alternative embodiment, the loquat kernel is used in ground form. In this embodiment, the kernel may have been ground to any suitable size. Preferably, the kernel has been ground to pass through a 5mm sieve, more preferably a 2mm sieve, most preferably a 1mm sieve.

**[0016]** However, it is preferred that the kernel has not been ground too finely, as such a material would decompose relatively rapidly and therefore would be less effective; it is therefore preferred that 50% or more of the ground kernel would not pass through a 0.05mm sieve; more preferably 60% or more, such as 70% or more, for example 80% or more. In one embodiment, the kernel has been ground such that 50% or more of the ground kernel will not pass through a 0.1mm sieve; more preferably 60% or more, such as 70% or more, for example 80% or more.

**[0017]** The loquat kernel may be used in any suitable amounts or concentrations; clearly the amount used should be selected appropriately depending upon the intended application.

**[0018]** When considered on a wt/v basis, the loquat kernel may suitably be used in concentrations of 10g/l or more, preferably 15g/l or more, more preferably 20g/l or more, most preferably 25g/l or more, for example 30g/l or more.

**[0019]** When considered on a wt/wt basis, the loquat kernel may suitably be used in concentrations of 10g/kg or more, preferably 15g/kg or more, more preferably 20g/kg or more, most preferably 25g/kg or more, for example 30g/kg or more.

**[0020]** The invention further provides, in a second aspect, a method of killing, inactivating or repelling nematodes, which method comprises providing loquat kernel and applying the loquat kernel to the nematodes or to an area where the nematodes are likely to exist or to an area to where the nematodes are expected to move.

**[0021]** Suitably the method comprises providing the loquat kernel in ground, chopped, freeze dried or spray dried form or in the form of an extract. When provided in the form of an extract any suitable solvent may be used; for example an aqueous solvent, such as water, or an organic solvent, such as ethanol, acetone or dichloromethane. In one embodiment, the kernel is provided in the form of an extract in water. In an alternative embodiment, the kernel is provided in the form of an extract in ethanol.

**[0022]** Suitably, all soluble components from the loquat kernel may be provided, in particular all water-soluble components and/or all ethanol-soluble components from the loquat kernel may suitably be provided.

**[0023]** In one embodiment the loquat kernel is provided in the form of an extract, such as an extract in water, from which all or substantially all volatiles have been removed.

**[0024]** In an alternative embodiment, the loquat kernel is provided in ground form.

**[0025]** The method may suitably comprise the steps of providing loquat kernel, taking an extract thereof and applying the loquat kernel extract to the nematodes or to an area where the nematodes are likely to exist or to an area to where the nematodes are expected to move.

**[0026]** In particular, the step of taking an extract of the loquat kernel may comprise taking an extract of the kernel with water or with ethanol.

**[0027]** The method may further comprise the step of removing all or substantially all volatiles from the extract before the step of applying the extract.

**[0028]** The method may alternatively comprise the steps of providing loquat kernel, grinding it and applying the ground loquat kernel to the nematodes or to an area where the nematodes are likely to exist or to an area to where the nematodes are expected to move.

**[0029]** The kernel may be ground to any suitable size. Preferably, the kernel is ground to pass through a 5mm sieve, more preferably a 2mm sieve, most preferably a 1mm sieve.

**[0030]** However, it is preferred that the kernel is not ground too finely, as such a material would decompose relatively rapidly and therefore would be less effective; it is therefore preferred that 50% or more of the ground kernel would not pass through a 0.05mm sieve; more preferably 60% or more, such as 70% or more, for example 80% or more. In one embodiment, the kernel is ground such that 50% or more of the ground kernel will not pass through a 0.1mm sieve; more preferably 60% or more, such as 70% or more, for example 80% or more.

**[0031]** The loquat kernel may be applied in any suitable amounts or concentrations; clearly the amount used should be selected appropriately depending upon the intended application.

**[0032]** When considered on a wt/v basis, the loquat kernel may suitably be applied in concentrations of 10g/l or more, preferably 15g/l or more, more preferably 20g/l or more, most preferably 25g/l or more, for example 30g/l or more.

**[0033]** When considered on a wt/wt basis, the loquat kernel may suitably be applied in concentrations of 10g/kg or more, preferably 15g/kg or more, more preferably 20g/kg or more, most preferably 25g/kg or more, for example 30g/kg or more.

**[0034]** Preferably, the nematodes are plant parasitic nematodes, for example root knot nematodes such as *Meloidogyne* spp.

**[0035]** It is preferred that the method is a method for selectively killing plant parasitic nematodes.

**[0036]** It is preferred that the method is a method for selectively killing nematodes over fungi.

**[0037]** The method may suitably involve applying loquat kernel to a growing medium, such as soil, peat, sand or water, in an area where nematodes are believed to exist or to a growing medium, such as soil, peat, sand or water, in an area to where nematodes are expected to move. For example, the loquat kernel may be applied to or mixed with a growing medium, such as soil, peat, sand or water, that is to have plants planted in it or may be applied to or mixed with a growing medium, such as soil, peat, sand or water, that has plants planted in it, especially when the plants are those that are vulnerable to nematodes.

**[0038]** The invention further provides, in a third aspect, a nematocide comprising loquat kernel and a pesticidally acceptable solvent, carrier, excipient or diluent.

**[0039]** The nematocide may suitably comprise all soluble components of loquat kernel, in particular all water-soluble components and/or all ethanol-soluble components of loquat kernel.

**[0040]** Suitably, the nematocide comprises, when considered on a wt/v basis, 10g/l of loquat kernel or more, preferably 15g/l or more, more preferably 20g/l or more, most preferably 25g/l or more, for example 30g/l or more.

**[0041]** When considered on a wt/wt basis, the nematocide suitably comprises 10g/kg of loquat kernel or more, preferably 15g/kg or more, more preferably 20g/kg or more, most preferably 25g/kg or more, for example 30g/kg or more.

**[0042]** The nematocide may comprise the kernel in ground, chopped, freeze dried or spray dried form or in the form of an extract. When in the form of an extract, any suitable solvent may be used, for example an aqueous solvent such as water or an organic solvent such as ethanol, acetone or dichloromethane. In one embodiment, the nematocide com-

prises an extract of loquat kernel in a pesticidally acceptable solvent, carrier, excipient or diluent. Preferably, the nematocide comprises an extract of loquat kernel in water.

[0043] In one particular such embodiment, the nematocide comprises an extract of loquat kernel, preferably an extract in water, that has a zero or substantially zero concentration of volatiles. In particular the nematocide may comprise an extract of loquat kernel, preferably an extract in water, that has had all or substantially all volatiles removed.

[0044] In an alternative embodiment, the nematocide comprises loquat kernel in ground form. In this embodiment, the kernel may have been ground to any suitable size. Preferably, the kernel has been ground to pass through a 5mm sieve, more preferably a 2mm sieve, most preferably a 1mm sieve.

[0045] However, it is preferred that the kernel has not been ground too finely, as such a material would decompose relatively rapidly and therefore would be less effective; it is therefore preferred that 50% or more of the ground kernel would not pass through a 0.05mm sieve; more preferably 60% or more, such as 70% or more, for example 80% or more. In one embodiment, the kernel has been ground such that 50% or more of the ground kernel will not pass through a 0.1mm sieve; more preferably 60% or more, such as 70% or more, for example 80% or more.

[0046] The present invention also provides, in a fourth aspect, a method of producing a nematocide, which method comprises providing ground, chopped, freeze dried or spray dried loquat kernel.

[0047] In one embodiment, the method comprises providing ground loquat kernel. In this embodiment, the kernel may have been ground to any suitable size. Preferably, the kernel has been ground to pass through a 5mm sieve, more preferably a 2mm sieve, most preferably a 1mm sieve.

[0048] However, it is preferred that the kernel has not been ground too finely, as such a material would decompose relatively rapidly and therefore would be less effective; it is therefore preferred that 50% or more of the ground kernel would not pass through a 0.05mm sieve; more preferably 60% or more, such as 70% or more, for example 80% or more. In one embodiment, the kernel has been ground such that 50% or more of the ground kernel will not pass through a 0.1mm sieve; more preferably 60% or more, such as 70% or more, for example 80% or more.

[0049] In one particular such embodiment, the step of providing ground loquat kernel comprises the sub steps of providing loquat kernel and then grinding the kernel.

[0050] In one embodiment, the method comprises providing ground loquat kernel and further comprises the step of taking extracts of the ground loquat kernel. Any suitable solvent may be used to take the extract, for example an aqueous solvent, such as water, or an organic solvent, such as ethanol, acetone or dichloromethane. In particular, the method may comprise the further step of taking extracts in water or extracts in ethanol of the ground loquat kernel.

[0051] The method may further comprise the step of removing all or substantially all volatiles from the ground kernel extract.

[0052] In one particular such embodiment, the step of providing ground loquat kernel comprises the sub steps of providing loquat kernel and then grinding the kernel.

[0053] The invention will now be further described, for the purposes of illustration only, in the following examples.

## EXAMPLES

[0054] Water extracts from Moraira loquat kernels (Algerie or Cardona varieties) were evaluated in a number of experiments. The stock solution used in each case was prepared by 5 g ground kernels (fresh weight) being extracted in Soxhlo<sup>TM</sup> with 50 ml distilled water.

### Example 1 - Experiments with Juvenile root-knot nematodes

[0055] The water extracts were tested against plant parasitic nematodes, *Meloidogyne* spp. This root knot nematode is a major crop pest.

[0056] Juvenile root-knot nematodes were exposed to different concentrations of the stock solution.

#### Method:

[0057] Two Stock Solutions were prepared as above. One was prepared and stored in the fridge for a month (Stock A) and the other was produced and used immediately (Stock B). Nematodes were then treated with 90, 50 and 10% concentrations of these Stock Solutions (e.g. for a 90% concentration the ratio of stock solution: water was 9:1, v/v). Equivalent controls were left in water.

[0058] After treatment the nematodes were placed in water to observe any recovery.

#### Results:

[0059] The product inactivated the nematodes immediately.

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| Root Knot Nematodes (using Stock B) |                                          |             |             |                         |
|-------------------------------------|------------------------------------------|-------------|-------------|-------------------------|
| Concentration                       | % Inactivation after initial application |             |             | Average Inactivation(%) |
|                                     | Replicate 1                              | Replicate 2 | Replicate 3 |                         |
| 90% solution                        | 100                                      | 100         | 100         | 100.0                   |
| 50% solution                        | 100                                      | 100         | 100         | 100.0                   |
| 10% solution                        | 100                                      | 100         | 99.5        | 99.8                    |
| 90% Control                         | 0                                        | 0           | 0           | 0.00                    |
| 50% Control                         | 0                                        | 0           | 0           | 0.00                    |
| 10% Control                         | 0                                        | 0           | 0           | 0.00                    |

| Root Knot Nematodes (Using Stock A) |                                          |     |     |                          |
|-------------------------------------|------------------------------------------|-----|-----|--------------------------|
| Concentration                       | % Inactivation after initial application |     |     | Average inactivation (%) |
| 90% solution                        | 100                                      | 100 | 100 | 100.0                    |
| 50% solution                        | 100                                      | 100 | 100 | 100.0                    |
| 10% solution                        | 99                                       | 100 | 100 | 99.5                     |
| 90% Control                         | 0                                        | 0   | 0   | 0.00                     |
| 50% Control                         | 0                                        | 0   | 0   | 0.00                     |
| 10% Control                         | 0                                        | 0   | 0   | 0.00                     |

**[0060]** When the nematodes were returned to fresh water and observed for 22 hours, only those that had been inactivated by the 10% solution recovered.

### Conclusion:

**[0061]** The solutions killed root-knot nematodes at 50% and 90% concentration, but at 10% inactivation was reversible.

### Example 2 - Further Experiments with Juvenile root-knot nematodes

#### Method:

**[0062]** Example 1 was repeated but using 40, 30, and 20% solutions.

#### Results:

**[0063]** The same results as in Example 1 were obtained. No recovery occurred when nematodes were replaced in water.

### Conclusion:

**[0064]** The concentration of Solution at which there is inactivation with no recovery is between 10 and 20%.

**[0065]** In further repeat studies the period of observation of recovery was extended to 8 days (instead of 22 hours as above). No significant recovery was recorded with the 40, 30, and 20% solutions.

### Example 3 - The efficacy of the product on inactivation of root-knot nematodes after storage.

#### Method:

**[0066]** Two different batches of Stock Solution prepared as above were tested to compare whether any loss of efficacy

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had occurred over time. Stock C was stored in the fridge at 5°C for a month after preparation; Stock D was freshly prepared from ground kernels that had been stored in the freezer before use. The time it took for the root-knot nematodes to become inactivated by the product was compared for the two batches. Two concentrations, 90% and 50%, and a water control were evaluated.

### Result:

[0067] Both concentrations of both Stock C and Stock D caused instantaneous paralysis to the root-knot nematodes.

### Conclusion:

[0068] Efficacy was not lost during storage of the product at 5°C.

### Example 4 - The role of the volatile compounds in the Moraira kernels

[0069] Experiments were carried out to determine whether volatile compounds in the kernels are the principles that kill the nematodes.

### Method:

[0070] Experiments were conducted under conditions as described above, but in one treatment lids of containers were removed (to allow volatiles to escape). Dosages were 50, 40, 30 and 20% of the above Stock Solution.

### Results:

[0071] The nematodes were observed over 3 days. In both treatments (with or without lid) and at all concentrations the nematodes ceased to move [indicated by (X)]. Controls remained active (data not shown)

| Experiment with lid |   |   |   |     |   |   |   |     |   |   |   |     |   |   |   |
|---------------------|---|---|---|-----|---|---|---|-----|---|---|---|-----|---|---|---|
| Day                 | 1 | 2 | 3 | Day | 1 | 2 | 3 | Day | 1 | 2 | 3 | Day | 1 | 2 | 3 |
| 50%                 | X | X | X | 40% | X | X | X | 30% | X | X | X | 20% | X | X | X |

| Experiment without lid |   |   |   |     |   |   |   |     |   |   |   |     |   |   |   |
|------------------------|---|---|---|-----|---|---|---|-----|---|---|---|-----|---|---|---|
| Day                    | 1 | 2 | 3 | Day | 1 | 2 | 3 | Day | 1 | 2 | 3 | Day | 1 | 2 | 3 |
| 50%                    | X | X | X | 40% | X | X | X | 30% | X | X | X | 20% | X | X | X |

### Conclusion:

[0072] The results suggest that the active principles are not all volatiles.

[0073] The work was then repeated using solutions from which the volatiles had been removed. The same results were shown, which suggests that there are non-volatile compounds in the water extract that will kill root-knot nematodes.

### Example 5 - The activity of the water extract against non-plant parasitic nematodes

[0074] Experiments were carried out to determine whether the water extracts are active on all types of nematodes, or whether they show some selectivity against different types of nematodes.

### Method:

[0075]

I. Nematodes that feed on bacteria are common soil inhabitants. Some of these nematodes of the genus *Steinernema* were exposed to a 50% solution of the Stock Solution.

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II. A field-collected mixed population of all trophic groups of nematodes (fungus-feeding, bacteria-feeding, predatory and plant parasitic) was exposed to a 50% solution of the Stock Solution.

### Results:

[0076]

I. The *Steinernema* nematodes were not inactivated.

II. Of the mixed population all the plant parasites were inactivated. Some of the non-plant parasites were still active, this probably varied across trophic groups.

### Conclusion:

[0077] Nematodes with different feeding behaviours are affected differently by the product. All plant parasitic nematodes were inactivated.

### Example 6 - Activity against larvae of the wax moth *Galleria mellonella*

[0078] The wax moth *Galleria mellonella* is a standard laboratory test insect and such moths are readily mass-produced in the laboratory or can be bought from a supplier, e.g. Live Foods Direct.

### Methods:

[0079] In several studies larvae (larvae either of different stages or only 1<sup>st</sup> instar larvae) were exposed to the product by direct application. Observations continued over 36 days.

### Results:

[0080] The product did not show activity when applied to the larvae. Development to adult progressed although in some preliminary tests the water extract appeared to halt development. The experiment as repeated with 1<sup>st</sup> instar larvae showed no effect.

### Example 7 - Activity against some beneficial insects

[0081] The wasp *Encarsia formosa*, parasitic on whiteflies, the predacious mite *Phytoseiulus persimilis* that preys on spider mites and nymphs and adults of the thrip predator *Amblyseius cucumeris* (all bought from a biological control company) were exposed to the Stock Solution.

### Results:

[0082] There was no mortality.

### Example 8 - Activity against a pathogenic fungus

### Method:

[0083] The Stock Solution was tested at full strength against two isolates of the root-infecting fungus *Pythium aphanideratum*.

[0084] Plugs of actively growing fungus were placed on water-agar in Petri plates; drops of 25 and 50 microlitres of the product were placed in different positions around the fungus. Growth of the fungus was monitored over 48 h at 25 °C. Sterile water drops were used as controls.

### Result:

[0085] Growth of the fungus was not inhibited.

### Example 9 - Activity testing of loquat kernels in a water screen

#### Method:

**[0086]** 100 microlitres containing nematodes were pipetted into 2.5 cm Petri dishes. The nematodes used were principally root-knot nematodes (*Meloidogyne* sp) but some initial observations were also made on some *Pratylenchus* sp. and free-living species (non-plant parasites).

**[0087]** 900 microlitres of Loquat extract, or 500 microlitres of Loquat extract plus 400 microlitres of water, were added to the nematodes to give concentrations of 90 or 50% of the original Loquat extract. Total added volume was therefore 1ml. Comparable experiments using amygdalin solution instead (solutions made from (a) 70 mg dissolved in 50 ml water and (b) 400 mg dissolved in 50 ml water) were also carried out.

**[0088]** Controls were nematodes from the same source placed in tap water.

**[0089]** Following application of the Loquat or amygdalin solution, observations were made after 10 minutes, the next day, and after 5 days.

#### Results:

##### 10 minutes after application:

**[0090]**

- No movement of plant parasitic nematodes observed at either concentration of Loquat kernels; but free-living nematodes remained active.

- Amygdalin: all nematodes still active at both concentrations after 10 minutes.

##### Next day:

**[0091]**

- No movement of the plant parasitic nematodes at either concentration of Loquat kernels.
- Nematodes in both amygdalin treatments were active.

##### 5 days later:

**[0092]** Same results: no movement in Loquat kernel extracts; but nematodes still active in both amygdalin treatments.

#### Conclusions:

**[0093]** Loquat kernels in the water screen cause plant parasitic nematodes to be killed, but free living, beneficial nematodes are not damaged.

**[0094]** However, amygdalin itself is not active *per se*.

### Example 10 - Pot experiments with nematodes and plants in greenhouse

#### Method:

**[0095]** Ground kernels were thoroughly mixed into John Innes No. 2 loam based compost (33 g). Doses applied were 0.33, 0.83, 1.66g per pot and an untreated control.

**[0096]** Four-week old tomato plants cv Tiny Tim were planted into the amended soil and approximately 700 root-knot nematode juveniles were then inoculated.

**[0097]** Plants were left to grow in a heated glasshouse at 20°C (+/- 5°C). After 48 days plants were washed from the pots; fresh weights of foliage and root systems were taken; numbers of root-knot nematode egg masses per root system were recorded.

#### Results:

**[0098]**

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| Treatment level |      | Root weight | Shoot weight | Gall index | Total egg masses | Egg masses/g root |
|-----------------|------|-------------|--------------|------------|------------------|-------------------|
| Control         | 1    | 1.84        | 3.45         | 4          | 156              | 84.78             |
|                 | 2    | 2.12        | 2.58         | 4          | 178              | 83.96             |
|                 | 3    | 1.80        | 2.51         | 4          | 161              | 89.44             |
|                 | 4    | 1.47        | 2.33         | 4          | 197              | 134.01            |
|                 | Mean | 1.81        | 2.72         | 4.0        | 173              | 98.05             |
| 0.33g           | 1    | 2.51        | 2.57         | 3          | 200              | 79.68             |
|                 | 2    | 2.07        | 3.08         | 4          | 198              | 95.65             |
|                 | 3    | 3.04        | 2.01         | 3          | 89               | 29.25             |
|                 | 4    | 2.76        | 2.86         | 4          | 118              | 42.75             |
|                 | Mean | 2.60        | 2.63         | 3.5        | 151              | 61.83             |
| 0.83g           | 1    | 1.28        | 2.09         | 3          | 104              | 81.25             |
|                 | 2    | 2.16        | 2.22         | 4          | 112              | 51.85             |
|                 | 3    | 1.74        | 2.39         | 4          | 68               | 39.08             |
|                 | 4    | 1.77        | 2.48         | 4          | 140              | 79.09             |
|                 | Mean | 1.74        | 2.30         | 3.8        | 106              | 62.82             |
| 1.66g           | 1    | 0.81        | 2.14         | 2          | 38               | 46.91             |
|                 | 2    | 1.43        | 1.93         | 3          | 30               | 20.98             |
|                 | 3    | 1.75        | 2.75         | 2          | 28               | 16.00             |
|                 | 4    | 1.72        | 2.48         | 2          | 98               | 56.98             |
|                 | Mean | 1.43        | 2.33         | 2.3        | 49               | 35.22             |

### Conclusions:

**[0099]** Significantly fewer egg masses were produced on plants treated with the highest dose. This would confirm that there are nematicidal compounds in the Loquat seeds.

**[0100]** The product also appears somewhat phytotoxic and this was reflected in lower root weights and in the slightly stunted shoots.

### Example 11 - Effect of ground kernels on nematodes in soil

#### Method:

**[0101]** 30cm<sup>3</sup> of air dried John Innes loam based compost was treated with 0.11, 0.28 and 0.55 g of ground kernel in plastic containers. Each was then inoculated with 600 root-knot nematodes contained in 1ml water and a further 7ml water was added to provide adequate moisture. After 48h nematodes were extracted from the soil using the conventional incubation technique over 24 h. The treatments had four replicates.

#### Results:

**[0102]** Figure 1 is a graph showing the results for the different levels of treatment.

### Conclusions:

**[0103]** All Loquat treatments had a significant effect on the recovery of nematodes from the soil indicating that the kernel powder had inactivated the nematodes during the 48h period.

**Example 12 - Dipping tomato plants in aqueous loquat extract**

**Method:**

5   **[0104]** Two-week old tomato plants were dipped for 1h in Loquat extracts of 25, 50, and 75% of the original stock material. The treated plants were then planted into soil (John Innes No2 loam-based compost) and inoculated with 375 root-knot nematodes. The plants were then grown in the glasshouse for 1 month when nematode development was assessed.

10   **Result:**

**[0105]** Figure 2 is a graph showing the results for the different levels of treatment.

**Conclusions:**

15   **[0106]** The effect of dipping the tomato root systems in all concentrations of the extract prevented invasion by the root-knot nematodes. This suggests that the toxic effects of the extract were sufficiently persistent to prevent nematodes invading.

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**Claims**

1. The use of loquat kernel to kill, inactivate or repel nematodes.
- 25   2. Use according to Claim 1, wherein the use is as a nematicide.
3. Use according to Claim 1 or Claim 2, wherein the use is against plant parasitic nematodes.
4. Use according to Claim 3, wherein the use is as a selective nematicide for plant parasitic nematodes.
- 30   5. Use according to any one of Claims 1 to 4, wherein the use is as a selective treatment for nematodes over fungi.
6. Use according to any one of the preceding claims wherein the loquat kernel is used in ground, chopped, freeze dried or spray dried form or in the form of an extract.
- 35   7. Use according to Claim 6, wherein the kernel is used in the form of an extract in water.
8. Use according to Claim 6, wherein the kernel is used in the form of an extract in an organic solvent.
- 40   9. Use according to Claim 8, wherein the solvent is ethanol.
10. Use according to any one of the preceding claims, wherein all soluble components from the loquat kernel are used.
11. Use according to any one of the preceding claims, wherein all water-soluble components from the loquat kernel are used.
- 45   12. Use according to any one of the preceding claims, wherein all organic solvent-soluble components from the loquat kernel are used.
13. Use according to any one of the preceding claims, wherein all ethanol-soluble components from the loquat kernel are used.
- 50   14. Use according to Claim 6, wherein the loquat kernel is in ground form.
15. Use according to Claim 14, wherein the kernel has been ground so as to pass through a 5mm sieve but such that 50% or more of the ground kernel would not pass through a 0.1mm sieve.
- 55   16. A method of killing, inactivating or repelling nematodes, which method comprises providing loquat kernel and applying

the loquat kernel to the nematodes or to an area where the nematodes are likely to exist or to an area to where the nematodes are expected to move.

5 17. The method of Claim 16 wherein the method comprises providing the loquat kernel in ground, chopped, freeze dried or spray dried form or in the form of an extract.

18. The method of Claim 17, wherein the method comprises the kernel in the form of an extract in water.

10 19. The method of Claim 17, wherein the method comprises the kernel in the form of an extract in an organic solvent.

20. The method according to Claim 19, wherein the solvent is ethanol.

15 21. The method according to any one of Claims 16 to 20, wherein all soluble components from the loquat kernel are provided.

22. The method of Claim 17 wherein the loquat kernel is provided in ground form.

23. The method of any one of Claims 16 to 22 wherein the nematodes are plant parasitic nematodes.

20 24. The method of Claim 23 wherein the nematodes are root knot nematodes.

25 25. The method of any one of Claims 16 to 24 wherein the method comprises applying loquat kernel to a growing medium in an area where nematodes are believed to exist or to a growing medium in an area to where nematodes are expected to move.

26. The method of Claim 25 wherein the loquat kernel is applied to or mixed with a growing medium that is to have plants planted in it or is applied to or mixed with a growing medium that has plants planted in it.

30 27. A nematicide comprising loquat kernel and a pesticidally acceptable solvent, carrier, excipient or diluent.

28. The nematicide of Claim 27, which comprises all soluble components of loquat kernel.

29. The nematicide of Claim 27, which comprises all water-soluble components of loquat kernel.

35 30. The nematicide of Claim 27, which comprises all ethanol-soluble components of loquat kernel.

31. The nematicide of any one of Claims 27 to 30, which comprises 10g/l of loquat kernel or more.

40 32. The nematicide of any one of Claims 27 to 31 wherein the nematicide comprises loquat kernel in ground, chopped, freeze dried or spray dried form or in the form of an extract.

33. A method of producing a nematicide, which method comprises providing ground, chopped, freeze dried or spray dried loquat kernel.

45 34. The method of Claim 33, which comprises providing ground loquat kernel.

35. The method of Claim 34, which further comprises taking extracts of the ground loquat kernel.

50 36. The method of Claim 35, wherein the extract is in water.

37. The method of Claim 35, wherein the extract is in ethanol.

## Patentansprüche

55 1. Die Verwendung von Wollmispelkernen zur Tötung, Inaktivierung oder Abwehr von Nematoden.

2. Verwendung gemäß Anspruch 1, wobei die Verwendung als Nematizid erfolgt.

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3. Verwendung gemäß Anspruch 1 oder Anspruch 2, wobei die Verwendung gegen parasitische Nematoden bei Pflanzen erfolgt.
- 5 4. Verwendung gemäß Anspruch 3, wobei die Verwendung als selektives Nematozid gegen parasitische Nematoden bei Pflanzen erfolgt.
5. Verwendung gemäß einem der Ansprüche 1 bis 4, wobei die Verwendung als selektive Behandlung von Nematoden bei Pilzen erfolgt.
- 10 6. Verwendung gemäß einem der vorstehenden Ansprüche, wobei die Wollmispelkerne in gemahlener, gehackter, gefriergetrockneter oder sprühgetrockneter Form oder in Form eines Extrakts verwendet werden.
7. Verwendung gemäß Anspruch 6, wobei die Kerne in Form eines Extrakts in Wasser verwendet werden.
- 15 8. Verwendung gemäß Anspruch 6, wobei die Kerne in Form eines Extrakts in organischem Lösungsmittel verwendet werden.
9. Verwendung gemäß Anspruch 8, wobei das Lösungsmittel Ethanol ist.
- 20 10. Verwendung gemäß einem der vorstehenden Ansprüche, wobei alle löslichen Bestandteile der Wollmispelkerne verwendet werden.
11. Verwendung gemäß einem der vorstehenden Ansprüche, wobei alle wasserlöslichen Bestandteile der Wollmispelkerne verwendet werden.
- 25 12. Verwendung gemäß einem der vorstehenden Ansprüche, wobei alle im organischen Lösungsmittel löslichen Bestandteile der Wollmispelkerne verwendet werden.
- 30 13. Verwendung gemäß einem der vorstehenden Ansprüche, wobei alle in Ethanol löslichen Bestandteile der Wollmispelkerne verwendet werden,
14. Verwendung gemäß Anspruch 6, wobei die Wollmispelkerne in gemahlener Form vorliegen.
- 35 15. Verwendung gemäß Anspruch 14, wobei die Kerne so gemahlen worden, dass sie durch ein 5-mm-Sieb hindurchgehen, jedoch so, dass 50% oder mehr der gemahlenden Kerne nicht durch ein 0,1-mm-Sieb hindurchgehen würden.
- 40 16. Verfahren zur Tötung, Inaktivierung oder Abwehr von Nematoden, wobei die Methode die Bereitstellung von Wollmispelkernen und die Anwendung von Wollmispelkernen gegen Nematoden oder in einem Bereich, in dem Nematoden gewöhnlich vorkommen oder in einem Bereich, wo sich Nematoden vermutlich bewegen, umfasst.
17. Verfahren nach Anspruch 16, wobei die Methode die Bereitstellung von Wollmispelkernen in gemahlener, gehackter, gefriergetrockneter oder sprühgetrockneter Form oder in Form eines Extrakts umfasst.
- 45 18. Verfahren nach Anspruch 17, wobei die Methode die Kerne in Form eines Extrakts in Wasser umfasst.
19. Verfahren nach Anspruch 17, wobei die Methode die Kerne in Form eines Extrakts in organischem Lösungsmittel umfasst.
- 50 20. Verfahren nach Anspruch 19, wobei das Lösungsmittel Ethanol ist.
21. Verfahren gemäß einem der Ansprüche 16 bis 20, wobei alle löslichen Bestandteile der Wollmispelkerne bereitgestellt werden.
- 55 22. Verfahren nach Anspruch 17, wobei die Wollmispelkerne in gemahlener Form bereitgestellt werden.
23. Verfahren nach einem der Ansprüche 16 bis 22, wobei die Nematoden parasitische Nematoden bei Pflanzen sind.
24. Verfahren nach Anspruch 23, wobei die Nematoden Wurzelgallennematoden sind.

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25. Verfahren nach einem der Ansprüche 16 bis 24, wobei die Methode die Anwendung der Wollmispelkerne an einem wachsenden Medium in einem Bereich, wo Nematoden gewöhnlich vorkommen, oder an einem wachsenden Medium in einem Bereich, in dem sich Nematoden vermutlich bewegen, umfasst.

26. Verfahren nach Anspruch 25, wobei die Wollmispelkerne auf ein Nährmedium aufgebracht oder mit diesem vermischt werden, um Pflanzen darin einzupflanzen, oder wobei die Wollmispelkerne auf ein Nährmedium aufgebracht oder mit einem Nährmedium vermischt werden, in das Pflanzen eingepflanzt sind.

27. Nematozid, das Wollmispelkerne und ein Lösungsmittel, eine Trägersubstanz, eine Transportsubstanz oder ein Verdünnungsmittel umfasst, das oder die für Pestizide geeignet ist.

28. Nematozid aus Anspruch 27, das alle löslichen Bestandteile der Wollmispelkerne umfasst.

29. Nematozid aus Anspruch 27, das alle wasserlöslichen Bestandteile der Wollmispelkerne umfasst.

30. Nematozid aus Anspruch 27, das alle in Ethanol löslichen Bestandteile der Wollmispelkerne umfasst.

31. Nematozid aus einem der Ansprüche 27 bis 30, das 10 g/l oder mehr Wollmispelkerne umfasst.

32. Nematozid aus einem der Ansprüche 27 bis 31, wobei das Nematozid Wollmispelkerne in gemahlener, gehackter, gefriergetrockneter, sprühgetrockneter Form oder in Form eines Extrakts umfasst.

33. Verfahren zur Herstellung eines Nematozids, wobei diese Methode die Bereitstellung von gemahlenen, gehackten, gefriergetrockneten oder sprühgetrockneten Wollmispelkernen umfasst.

34. Verfahren nach Anspruch 33, das die Bereitstellung von gemahlenen Wollmispelkernen umfasst.

35. Verfahren nach Anspruch 34, das außerdem die Gewinnung von Extrakten aus den gemahlenen Wollmispelkernen umfasst.

36. Verfahren nach Anspruch 35, wobei die Extraktion in Wasser erfolgt.

37. Verfahren nach Anspruch 35, wobei die Extraktion in Ethanol erfolgt.

### Revendications

1. Utilisation du noyau de la bibace pour tuer, inactiver ou repousser les nématodes.

2. Utilisation selon la revendication 1, dans laquelle l'utilisation est en tant que nématocide.

3. Utilisation selon la revendication 1 ou 2, dans laquelle l'utilisation est contre les nématodes phytoparasitaires.

4. Utilisation selon la revendication 3, dans laquelle l'utilisation est en tant que nématocide sélectif pour les nématodes phytoparasitaires.

5. Utilisation selon l'une quelconque des revendications 1 à 4, dans laquelle l'utilisation est en tant que traitement sélectif des nématodes sur les champignons.

6. Utilisation selon l'une quelconque des revendications précédentes, dans laquelle le noyau de bibace est utilisé sous une forme moulue, hachée, lyophilisée ou séchée par pulvérisation ou sous la forme d'un extrait.

7. Utilisation selon la revendication 6, dans laquelle le noyau est utilisé sous la forme d'un extrait dans de l'eau.

8. Utilisation selon la revendication 6, dans laquelle le noyau est utilisé sous la forme d'un extrait dans un solvant organique.

9. Utilisation selon la revendication 8, dans laquelle le solvant est de l'éthanol.

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10. Utilisation selon l'une quelconque des revendications précédentes, dans laquelle tous les composants solubles du noyau de bibace sont utilisés.
- 5 11. Utilisation selon l'une quelconque des revendications précédentes, dans laquelle tous les composants hydrosolubles du noyau de bibace sont utilisés.
12. Utilisation selon l'une quelconque des revendications précédentes, dans laquelle tous les composants du noyau de bibace solubles dans un solvant organique sont utilisés.
- 10 13. Utilisation selon l'une quelconque des revendications précédentes, dans laquelle tous les composants du noyau de bibace solubles dans l'éthanol sont utilisés.
14. Utilisation selon la revendication 6, dans laquelle le noyau est utilisé sous une forme moulue.
- 15 15. Utilisation selon la revendication 14, dans laquelle le noyau a été moulu de façon à passer à travers un tamis de 5 mm, mais de sorte que 50 % ou plus du noyau moulu ne passeraient pas à travers un tamis de 0,1 mm.
16. Procédé pour tuer, inactiver ou repousser les nématodes, lequel procédé comprend la fourniture de noyau de bibace et l'application du noyau de bibace sur les nématodes ou sur une zone dans laquelle les nématodes sont susceptibles d'exister ou sur une zone vers laquelle on prévoit que les nématodes se déplaceront.
- 20 17. Procédé selon la revendication 16, dans lequel le procédé comprend la fourniture du noyau de bibace sous une forme moulue, hachée, lyophilisée ou séchée par pulvérisation ou sous la forme d'un extrait.
- 25 18. Procédé selon la revendication 17, dans lequel le procédé comprend le noyau sous la forme d'un extrait dans de l'eau.
19. Procédé selon la revendication 17, dans lequel le procédé comprend le noyau sous la forme d'un extrait dans un solvant organique.
- 30 20. Procédé selon la revendication 19, dans lequel le solvant est de l'éthanol.
21. Procédé selon l'une quelconque des revendications 16 à 20, dans lequel tous les composants solubles du noyau de bibace sont fournis.
- 35 22. Procédé selon la revendication 17, dans lequel le noyau de bibace est fourni sous forme moulue.
23. Procédé selon l'une quelconque des revendications 16 à 22, dans lequel les nématodes sont des nématodes phytoparasitaires.
- 40 24. Procédé selon la revendication 23, dans lequel les nématodes sont des nématodes des racines.
25. Procédé selon l'une quelconque des revendications 16 à 24, dans lequel le procédé comprend l'application du noyau de bibace à un milieu de croissance dans une zone où on pense que les nématodes existent ou à un milieu de croissance dans une zone vers laquelle on prévoit que les nématodes se déplaceront.
- 45 26. Procédé selon la revendication 25, dans lequel le noyau de bibace est appliqué ou mélangé à un milieu de croissance dans lequel des plantes seront plantées ou est appliqué ou mélangé à un milieu de croissance dans lequel des plantes sont plantées.
- 50 27. Nématocide comprenant du noyau de bibace et un solvant, support, excipient ou diluant acceptables sur le plan pesticide.
28. Nématocide selon la revendication 27, qui comprend tous les composants solubles du noyau de bibace.
- 55 29. Nématocide selon la revendication 27, qui comprend tous les composants hydrosolubles du noyau de bibace.
30. Nématocide selon la revendication 27, qui comprend tous les composants du noyau de bibace solubles dans l'éthanol.

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- 31.** Nématocide selon l'une quelconque des revendications 27 à 30, qui comprend 10 g/l de noyau de bibace ou plus.
- 32.** Nématocide selon l'une quelconque des revendications 27 à 31, dans lequel le nématocide comprend le noyau de bibace sous une forme moulue, hachée, lyophilisée ou séchée par pulvérisation, ou sous la forme d'un extrait.
- 33.** Procédé de fabrication d'un nématocide, lequel procédé comprend la fourniture de noyau de bibace moulu, haché, lyophilisé ou séché par pulvérisation.
- 34.** Procédé selon la revendication 33, qui comprend la fourniture de noyau de bibace moulu.
- 35.** Procédé selon la revendication 34, qui comprend en outre le prélèvement d'extraits du noyau de bibace moulu.
- 36.** Procédé selon la revendication 35, dans lequel l'extrait est dans de l'eau.
- 37.** Procédé selon la revendication 35, dans lequel l'extrait est dans de l'éthanol.

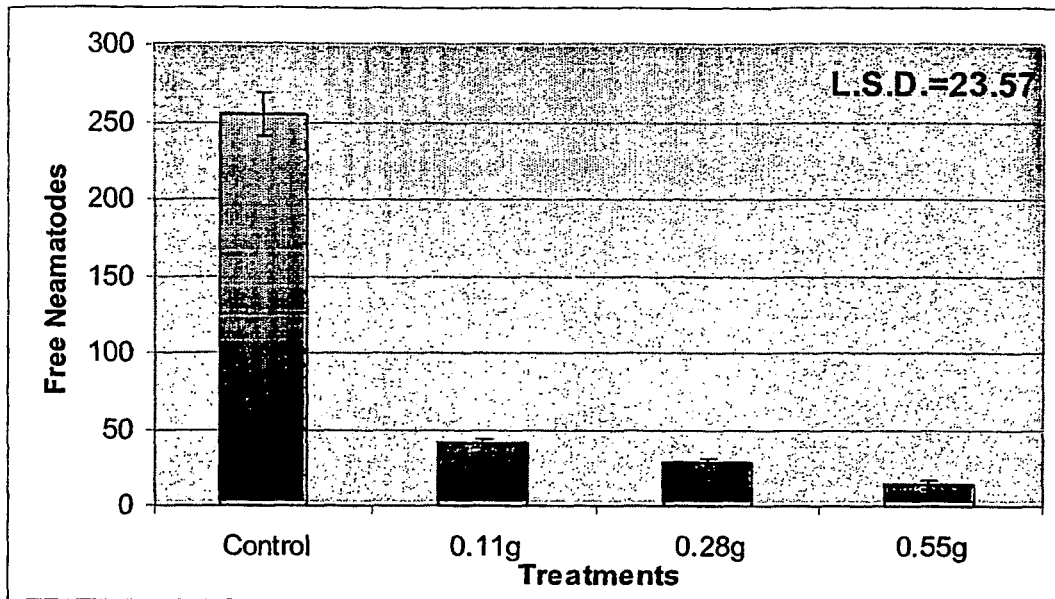


Fig 1

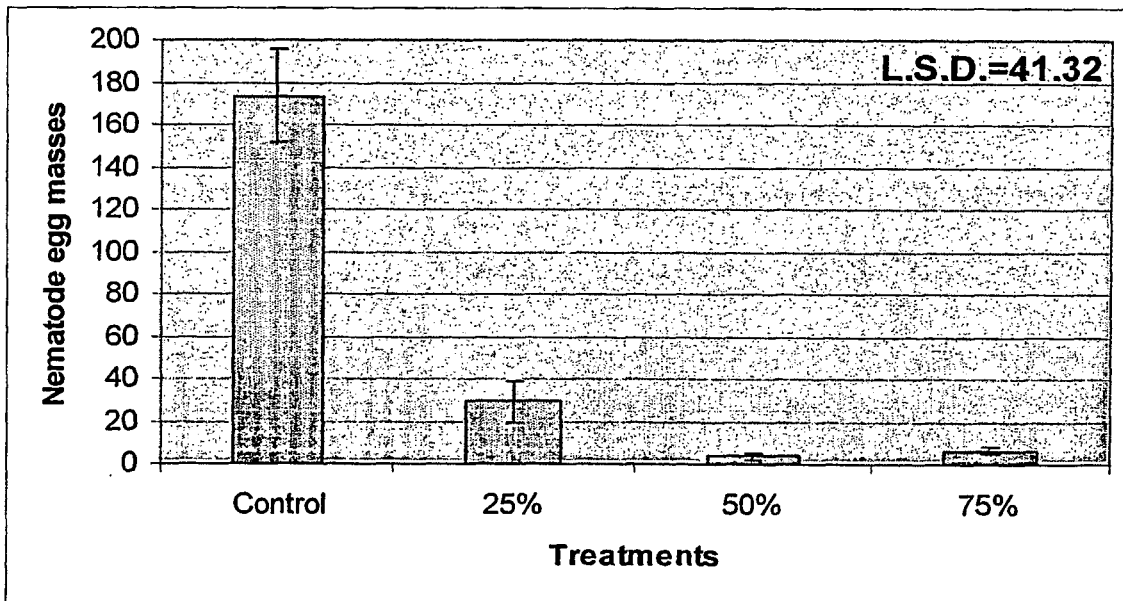


Fig. 2